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The influence of salinity on the heat-shock protein response of *Potamocorbula amurensis* (Bivalvia)

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Abstract

For biomarkers to be useful in assessing anthropogenic impacts in field studies involving aquatic organisms, they should not be affected by naturally occurring changes in environmental parameters such as salinity. This is especially important in estuarine environments and for relatively unspecific biomarkers like heat-shock proteins (hsps, stress proteins). In this study, the heat-shock protein response was measured in the euryhaline clam, *Potamocorbula amurensis*, after exposure to a range of salinities reflecting normal and extreme environmental conditions in Northern San Francisco Bay, California. The ability to raise cellular hsp70 levels in response to heat-shock was significantly impaired in *P. amurensis* collected from a low (0.5 ppt) salinity field site, and after 14 day exposure to low salinity in the laboratory.

Keywords: Stress proteins; Bivalves; *Potamocorbula amurensis*; San Francisco Bay; Salinity; Heat-shock response

1. Introduction

Biomarker responses are expected to be sublethal, quantifiable, and reliable for interpretation. The latter requirement poses the greatest challenge, especially when biomarkers are applied in field situations. We presently lack thorough knowledge of background levels, variation due to seasonal and physiological cycles, and interactions with other cellular processes for many biomarkers. In addition, there is a need for information on the effects of natural stressors on the magnitude of the biomarker response.

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Our objective in this study was to study the influence of salinity on stress protein induction in the euryhaline clam *Potamocorbula amurensis*. Earlier work performed in this laboratory indicated that in clams exposed in the laboratory to a range of salinities, cellular stress protein levels were significantly lower at low (0.3 ppt) salinity than at high (27 ppt) salinity (Werner & Hinton, 2000). The work presented in this paper expands our existing knowledge and highlights the importance of acquiring "baseline" information on how stress protein expression is influenced by natural variables.

2. Methods

2.1. Heat-shock

P. amurensis were collected at USGS station 8.1 (August 1998, 24 ppt/19 °C), and maintained in the laboratory in the dark at 24 ppt/19 °C. After 24 h, clams (9 per beaker, shell-length: 1–1.8 cm) were heat-shocked (36.5–38 °C/30 min) then transferred to control conditions. Duplicate samples were taken at 3, 6, 9, 12, 24, 48, 72 h and 7 days after heat-shock, snap-frozen and stored at −80 °C.

2.2. Salinity experiment 1

Clams were collected from three USGS sites in Northern San Francisco Bay, 6.1, 8.1 and 12.5, on December 9, 1998. Bottom temperature at collection sites was 10 °C and salinities were 0.1 ppt (6.1), 5.6 ppt (8.1) and 14.9 ppt (12.5). Clams were maintained in the dark in aerated water from collection sites at 10 °C until the next day. Subsets of clams were heat-shocked (37 °C/30 min) in aerated water from sampling sites then returned to 10 °C. Samples were taken immediately (time 0) and 1, 3 and 6 h after heat-shock. Few clams were available from station 8.1 therefore samples were taken 1 and 6 h after heat-shock.

2.3. Salinity experiment 2

Clams (1–1.5 cm shell length) were collected from site 6.1 (6.4 ppt/13.3 °C) on April 6, 2000. They were acclimatized to laboratory conditions (16:8 light/dark cycle, 15 °C) in aerated containers (48 h) then exposed to salinities of 0.5, 6.5, and 25 ppt for 14 days (3 replicates per treatment, 8 clams pre replicate). Clams were not fed. Water was aerated and exchanged every 3 days. After 14 days subsets of clams were heat-shocked (37 °C/30 min) at respective salinities, then transferred to 15 °C. Samples were taken immediately (time 0), 6 and 24 h after heat-shock.

2.4. Stress protein analysis

Hsp70 proteins were analyzed in whole clams using Western blotting techniques following the protocol described in Werner and Hinton (1999). Briefly, 50 μg total

protein/sample were separated by SDS–PAGE, then electroblotted onto Immobilon-P membrane (Millipore). A monoclonal antibody for hsp70 (1:500; MA3-001, Affinity Bioreagents Inc., Golden, CO) was used as probe. This antibody recognizes two hsp70 isoforms, hsp70–72 and hsp76–78, in *P. amurensis*. Bound antibody was visualized by a chemiluminescent substrate (CDP-Star; Tropix Inc., Bedford, MA), and quantified by densitometry.

3. Results and discussion

Hsp70–72 was induced quickly (<3 h) and remained significantly elevated over control levels for 7 days (Fig. 1). Similarly, Clegg et al. (1998) showed that hsp70 proteins in oysters remain elevated for at least 14 days after a 1 h heat-shock. Compared to the hsp70 response recorded in other organisms this is surprisingly long (Feige, Yahara, Morimoto, & Polla, 1996).

Clams collected from station 6.1 at a salinity of 0.5 ppt had very low levels of hsp70–72 and were unable to increase cellular hsp70–72 concentrations in response to heat-shock, while clams from station 8.1 (data not shown) and 12.5 collected at salinities of 5.6 and 14.9 ppt were able to raise a heat-shock response (Fig. 2(a)). Hsp70–72 levels in all heat-shocked and most control (3, 6 h) groups from site 12.5 were significantly ($p < 0.05$) higher than in clams from station 6.1. Levels in clams from station 8.1 were similar to treatment groups from station 12.5 and 6 h after heat-shock, significantly higher than in clams from site 6.1. Similar observations have been reported in oysters, where exposure to 16 ppt resulted in delayed accumulation of hsp70 (Gary Cherr, UC Davis, pers. comm.).

Significant differences in hsp70–72 levels were found between clams exposed in the laboratory to different salinities, but these differences were not as dramatic as in

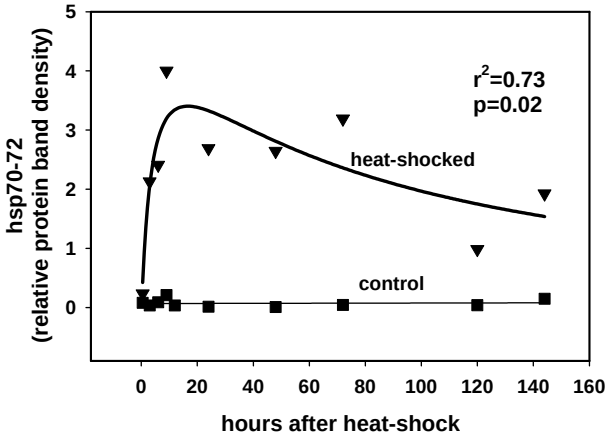

Fig. 1. Mean hsp70–72 levels in *P. amurensis* ($n = 2$) after a 30 min heat-shock at 36.5–38 °C. (■) Controls, (▼) Heat-shocked clams. The data follows a log normal (3 parameter) curve: $(f = 3.4 * \exp(-0.5 \ln(x/16.61)/1.71)^2)$.

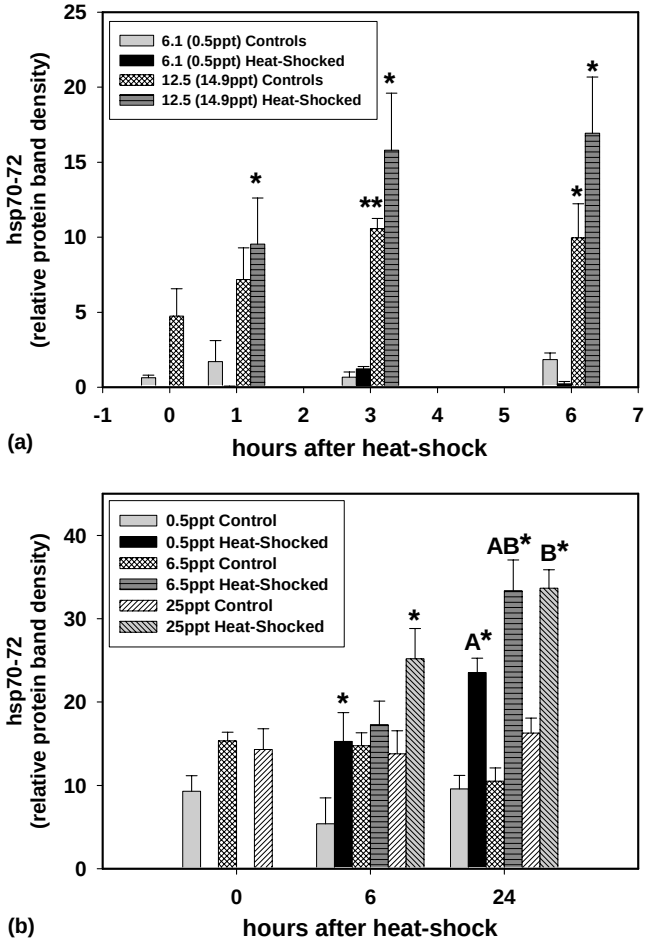

Fig. 2. Hsp70–72 response to heat-shock (37 °C, 30 min) in (a) *P. amurensis* collected from stations 6.1 (salinity: 0.5 ppt) and 12.5 (salinity: 14.9 ppt) in December 1998. *, ** indicate difference between sites 6.1 and 12.5 ($n = 3$) at significance levels $p < 0.05$ and $p < 0.001$, respectively. (b) *P. amurensis* exposed in the laboratory to different salinities (0.5, 6.5, 25 ppt) for 14 days. Shown are average band densities of western blots ± SE ($n = 3$). 0 h levels are the same for control and HS treatments. * Significantly different from control; A, B = groups are significantly different from each other ($p < 0.05$).

clams collected from different field sites. Hsp70–72 was inducible in all groups (Fig. 2(b)). Levels in animals exposed to 6.5 ppt were neither significantly different from those of 0.5 nor 25 ppt treated clams, although average hsp70–72 levels were similar to clams exposed to 25 ppt. The discrepancy between salinity experiments 1 (Fig. 2(a)) and 2 (Fig. 2(b)) may be due to the constant salinities clams were exposed to in the laboratory experiment, while clams in the field are subject to tidal fluctuations and have to constantly adapt to changing salinities.

There is little information on the mechanism underlying the observed attenuation of the heat-shock protein response. Previous studies on *P. amurensis* found significantly reduced hsp70 levels, low condition indices, low ATP and glycogen levels in clams collected from San Francisco Bay stations where salinity regularly reaches 0 ppt (sites 4.1 and 6.1) (Clark, Teh, & Hinton, 2000; Teh, Clark, Brown, Luoma, & Hinton, 1999; Werner & Hinton, 1999, 2000). Energy is not only needed for protein synthesis (Hofman & Somero, 1995), but also for the proper function of heat-shock proteins (Roberts, Hofmann, & Somero, 1997), and a link between reduced ATP levels and impaired stress protein function has previously been established (Feige et al., 1996). It is therefore hypothesized that energy depletion due to increased osmoregulation under low salinity conditions may lead to reduced levels of hsp70 proteins in clams.

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